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Impact of dietary nitrate supplementation on exercise capacity and cardiovascular parameters in chronic respiratory disease: a systematic review and meta-analysis

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ABSTRACT

Background Dietary nitrate supplementation, usually in the form of beetroot juice, may improve exercise performance and endothelial function. We undertook a systematic review and meta-analysis to establish whether this approach has beneficial effects in people with respiratory disease.

Methods A systematic search of records up to March 2021 was performed on PubMed, CINAHL, MEDLINE (Ovid), Cochrane and Embase to retrieve clinical trials that evaluated the efficacy of dietary nitrate supplementation on cardiovascular parameters and exercise capacity in chronic respiratory conditions. Two authors independently screened titles, abstracts and full texts of potential studies and performed the data extraction.

Results After full-text review of 67 papers, eleven (two randomised controlled trials and nine crossover trials) involving 282 participants met the inclusion criteria. Three were single dose; seven short term; and one, the largest (n=122), done in the context of pulmonary rehabilitation. Pooled analysis showed that dietary nitrate supplementation reduced systolic blood pressure (BP), diastolic BP and mean arterial pressure (mean difference (95% CI), −3.39 mm Hg (−6.79 to 0.01); p=0.05 and −2.20 mm Hg (−4.36 to −0.03); p=0.05 and −4.40 mm Hg (−7.49 to −1.30); p=0.005, respectively). It was associated with increased walk distance in the context of pulmonary rehabilitation (standardised mean difference (95% CI), 0.47 (0.11 to 0.83), p=0.01), but no effect was identified in short-term studies (0.08 (−0.32 to 0.49).

Conclusion Dietary nitrate supplementation may have a beneficial effect on BP and augment the effect of pulmonary rehabilitation on exercise capacity. Short-term studies do not suggest a consistent benefit on exercise capacity.

PROSPERO registration number CRD42019130123.

Key messages

- Does dietary nitrate supplementation improve cardiovascular parameters and exercise capacity in people with chronic respiratory disease?
- We found moderate evidence to support the hypothesis that dietary nitrate supplementation lowers blood pressure. There was low Grading of Recommendations, Assessment, Development and Evaluations evidence to support an improvement in exercise capacity in people with chronic obstructive pulmonary disease.

Why read on?

- This review systematically evaluates the available evidence regarding the impact of dietary nitrate supplementation on exercise capacity and cardiovascular parameters in individuals with respiratory disease.

INTRODUCTION

Exercise limitation is a common feature in individuals with chronic respiratory disease (CRD) despite optimum medical treatment including pulmonary rehabilitation (PR) and pharmacotherapy.[1–3] Factors contributing to breathlessness and reduced physical activity include altered pulmonary mechanics and cardiovascular function as well as skeletal muscle impairment.[4–7] Nitric oxide (NO) is a ubiquitous signalling molecule with a key role in endothelial function, and a relationship between plasma nitrite (NO_2^-) levels and exercise performance has been identified.[8 9] Dietary NO_3^- supplementation, which increases NO availability via a NO_3^-–NO_2^-–NO pathway, has therefore been proposed as a potential complementary approach to improve exercise capacity in people with cardiorespiratory disease.

In healthy adults, endurance exercise capacity increases following dietary NO_3^- supplementation[10 11] and evidence suggests that NO_3^- supplementation with beetroot juice (BRJ) can reduce oxygen consumption

(VO_2) during submaximal exercise and increase the time to reach exhaustion during high-intensity training.[12–14] Of note, dietary NO_3^- supplementation has been shown to improve exercise performance under hypoxic conditions,[15] and there is evidence for an effect in some clinical conditions, for example, chronic obstructive pulmonary disease (COPD),[16–19] peripheral vascular disease[20] and heart failure.[21 22]

In addition to exercise limitation, vascular comorbidities are common in people with lung disease, and clinical guidelines highlight the need to identify and optimally treat them.[23] If dietary NO_3^- supplementation can be shown to have a beneficial effect on exercise capacity and/or vascular comorbidities, it has the potential to improve outcomes in this patient group.

We therefore aimed to evaluate the available evidence regarding the impact of NO_3^- supplementation on exercise capacity and cardiovascular parameters in individuals with respiratory disease.

METHODS

The review was registered in the International Prospective Register of Systematic Reviews database. It was conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.

Search strategy

The first author (ASA) searched PubMed, CINAHL, MEDLINE (Ovid), Cochrane and Embase from inception to March 2021. The terms used in this search were 'respiratory diseases', 'chronic obstructive pulmonary disease', 'COPD', 'chronic obstructive airways disease', 'emphysema', 'bronchitis', 'bronchiectasis', 'interstitial lung disease', 'ILD', 'cystic fibrosis', 'pulmonary hypertension', 'PHT', 'nitrate', 'beetroot', 'dietary nitrate' and 'nitrate supplementation'. A search filter was applied by using medical subject heading terms. This search strategy was conducted in collaboration with a librarian to ensure this review contained the appropriate and necessary keywords. Full search strategy from all databases is presented in online supplemental appendix 1.

Inclusion criteria

We included both randomised clinical trials and crossover studies.

The PICO format was used in our search strategy

P: Population included adults diagnosed with CRD such as COPD, interstitial lung disease (ILD), bronchiectasis or pulmonary hypertension (PHT) either undergoing usual care or taking part in PR.

I: Intervention was dietary NO_3^- supplementation delivered to patients with CRD.

C: Comparator was a placebo group for interventional studies.

O: Outcomes included both primary outcomes (exercise capacity and blood pressure) and secondary outcomes such as cardiovascular parameters: heart rate (HR), oxygen saturation (O_2 sat), plasma NO_3^- and NO_2^- levels, peak and iso-time VO_2, endothelial function (flow-mediated dilatation (FMD)) and fractional exhaled NO (FeNO).

Exclusion criteria

1. Studies examining children under 18 years.
2. Any papers written in a language other than English.
3. Conference abstracts or unpublished data.

Data extraction

Data were extracted into a standardised Microsoft Excel spreadsheet form (Microsoft Corp., Redmond, WA, USA). We contacted the corresponding authors for missing data. Two authors (ASA and AMA) independently screened titles and abstracts of potential studies. A third reviewer (NSH) was available to resolve any disagreements. The form included data about change in exercise capacity following dietary NO_3^- supplementation. Other data such as cardiovascular parameters (HR, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and O_2 sat), VO_2, FMD, FeNO, intervention protocol (eg, dose, duration and delivery method), exercise protocol (type and duration) and placebo were extracted.

Data analysis

The synthesis of results described the outcomes of interest, such as exercise capacity, VO_2, exercise endurance time, plasma NO_3^- level, plasma NO_2^- level, FeNO, SBP, DBP, MAP, HR, O_2 sat and FMD. Where appropriate, meta-analysis was conducted to estimate the pooled differences and 95% CIs in the outcomes of interests between NO_3^- and placebo conditions. For crossover studies, endpoint values were extracted from the end of the study after receiving the supplements (NO_3^- or placebo) and included in meta-analysis as if from a parallel designed trial.[24 25] The random-effect model was applied because of the variety in several factors (eg, exercise protocol, dose and duration of NO_3^- supplementation). Continuous data are reported as the mean difference (MD) (Δ). Standardised mean difference (SMD) was used when the same outcome was assessed with different measures (eg, exercise capacity assessed using different walking tests). Heterogeneity among included studies was evaluating using the I^2 value. The statistical analyses were performed using the Cochrane Collaboration's Review Manager software (RevMan V.5.4.0).

RESULTS

Selection of studies

Initially, 2113 articles were identified through the database searches, 1554 after removing duplicates with 67 articles eligible for full-text review following title and abstract screening. Following full-text review, 11 studies met the inclusion criteria for the present systematic review (figure 1).

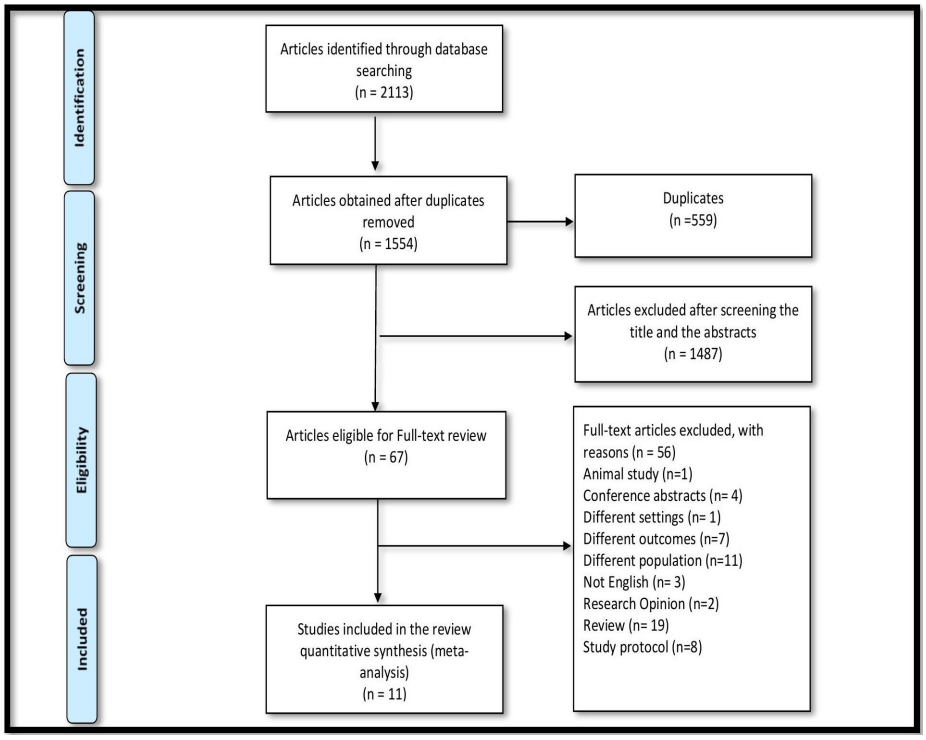

Figure 1 Flowchart of study selection (Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram).

Study characteristics

Among the 11 randomised controlled trials (RCT), two used a parallel design, and nine used a crossover design. All 11 were published between 2015 and 2020. Eight studies were conducted in Europe, two in the USA and one in Australia. Studies were categorised based on the reported duration of NO_3^- supplementation as 'acute effect' (single dose of NO_3^- supplementation, 2.5–3 hours before exercise session) (n=3), 'short term' (less than 3 months in usual care) (n=7) or 'during pulmonary rehabilitation' (n=1). One study provided both acute effect and short-term data.[26] The total number of participants was 282, including 15 with pulmonary arterial hypertension[27] and 267 with COPD[16–19 26 28–32] with sample sizes ranging from 8 to 122. Age of participants (mean±SD) was 66±3 years, and the majority (57%) were men. Ten trials used BRJ as the source of NO_3^- (n=10), and one used sodium NO_3^- ($NaNO_3^-$).[26] The dose of NO_3^- used ranged from 6.45 mmol[28] to 16 mmol[27]. A full description of the included studies is presented in table 1.

Exercise capacity

Data on exercise capacity or endurance were reported in ten studies using different tests including the incremental shuttle walk test (ISWT) (n=3), 6-minute walk distance (6MWD) (n=3), endurance time during cycle ergometry (n=3) and endurance shuttle walk test (ESWT) (n=1) in individuals with COPD[16–19 26 29–32] and PHT.[27]

The impact of NO_3^- supplementation on peak exercise capacity measured using walking tests (ISWT and 6MWD) in people with COPD is shown in (figure 2). Pooled analysis from five trials demonstrated an improvement in exercise capacity following NO_3^- supplementation compared with placebo (SMD (95% CI), 0.30 (0.03 to 0.57), p=0.03),[17–19 30 32] although this effect was driven by the study in the context of PR. Thus, supplementation was associated with increased walk distance in the context of PR (SMD (95% CI), 0.47 (0.11 to 0.83), p=0.01), but no effect was identified in short-term studies (0.08 (−0.32 to 0.49). A single trial in 15 patients with PHT taking BRJ for 1 week did not show a significant effect on 6MWD.[27]

Berry *et al* found an improvement in endurance exercise capacity during cycle ergometry at 75% maximal workload.[16] In contrast, results from two trials providing NO_3^- supplementation (one using BRJ and one $NaNO_3^-$) did not demonstrate a significant improvement in endurance exercise time during cycle ergometry.[26 29]

Similarly, Leong *et al* investigated the effect of 3 days of BRJ on exercise endurance via ESWT at 85% VO_2 max, among patients with stable moderate COPD, and found no difference in exercise endurance following BRJ compared with placebo.[31]

Effect of NO_3^- supplementation on physiological and cardiovascular parameters

Oxygen consumption

The impact of NO_3^- supplementation on peak VO_2 was reported in six studies (figure 3).[16 26–30] Pooled analysis from four trials demonstrated that peak VO_2 did not change following NO_3^- supplementation compared with placebo (MD (95% CI), 0.09 mL/min/kg (−1.36 to 1.53), p=0.91).[16 26 28 29] However, VO_2 at iso-time

Table 1 Description of the included studies

Authors/design	Study sample	Nitrate (NO_3^-) dose	Placebo	Duration	Wash-out	Results (following NO_3^- vs placebo)
Behnia *et al*, 2018/RCT[28]	N=25 GOLD stage 1–4 Age (y): 68±9 Sex (M/F): 13/12	70 mL BRJ + 180 mL black currant juice	70 mL water + 180 mL black currant juice	8 days	No	▶ No effect on VO_2 at peak (p>0.05) ▶ Significant change in SBP: 11 mm Hg (p<0.05) at peak exercise in the NO_3^- group only compared with pre-NO_3^- ▶ Significant increase in FeNO (ppb) in NO_3^- group (p<0.05) ▶ No effect on DBP (p>0.05) ▶ No effect on heart rate (HR) (p>0.05)
Beijers *et al*, 2018/RXT[26]	N=18 GOLD stage 1–2 Age (y): 67±8 Sex (M/F): 13/5 FEV_1%=69.2	Sodium NO_3^- (8 mmol)	NaCl ingestion	Acute and 7 days	7 days	▶ No effect on endurance cycle time (p=0.08) ▶ No effect on VO_2 on day 1 and day 7 during submaximal cycling at 70% Wmax (p=0.56) ▶ Significant increase in plasma NO_3^- level on day 1 and day 7 (p=<0.001) ▶ Significant increase on NO_2^- level on day 1 (p<0.001) and day 7 (p=0.003) ▶ No effect on SBP on day 1 and day 7 at 150 min (p=0.66) ▶ No effect on DBP on day 1 and day 7 at 150 min (p=0.35) ▶ No effect on physical activity, counts/min (p=0.53) ▶ No effect on HR on day 1 and day 7 at 150 min (p=0.76)
Berry *et al*, 2015/RXT[16]	N=15 GOLD stage 1–2 Age (y): 70±9 Sex (M/F): 12/3 FEV_1%=61.8	140 mL BRJ (7.58 mmol)	163 mL prune juice (0.01 mmol NO_3^-)	Acute	7 days	▶ 28.8 s longer in endurance cycle time (p=0.031) ▶ No effect on VO_2 at end exercise (p=0.43) ▶ Significant increase in plasma NO_3^- level (p<0.001) ▶ Significant increase in plasma NO_2^- level (p=0.001) ▶ Significant change in resting SBP: 8.2 mm Hg, p=0.019 ▶ Significant change in iso-time DBP: 6.4 mm Hg (p=0.001) ▶ Significant change end exercise in DBP: 5.6 mm Hg (p=0.08) ▶ No effect on HR, p=0.70 ▶ No effect on O_2 saturation, p=1.0
Curtis *et al*, 2015/RXT[29]	N=21 GOLD stage 2–4 Age (y): 68±7 Sex (M/F): 16/5 FEV_1%=50.1	140 mL BRJ (12.9 mmol)	140 mL ND-BRJ	Acute	7 days	▶ No effect on median endurance time (p=0.50) ▶ Significantly lower iso-time VO_2 (p=0.04) ▶ Significant increase in plasma NO_3^- level (p<0.0001) ▶ No effect on SBP ▶ Significant change in DBP: 7 mm Hg (p=0.01) ▶ No effect on MAP (p=0.07) ▶ No effect on HR (p=0.06) ▶ No effect on O_2 saturation (p=0.26)
Friis *et al*, 2015/RXT[30]	N=15 GOLD stage 2–4 Age (y): 63±13 Sex (M/F): 9/6 FEV_1%=44.7	140 mL BRJ	140 mL ND-BRJ	7 days	7 days	▶ No effect on exercise capacity (p=0.46) ▶ No effect on VO_2 (p=0.31) ▶ Significant increase in plasma NO_2^- level (p<0.01) ▶ No effect on SBP (p=0.80) ▶ Significant reduction on DBP (p<0.05) ▶ No effect on HR (p=0.86) ▶ No effect on physical activity (p>0.05)
Henrohn *et al*, 2018/RXT[27]	N=15 Pulmonary hypertension, WHO group 1 Age (y): 60±15 Sex (M/F): 7/8	140 mL BRJ (16 mmol)	140 mL ND-BRJ (0.118 mmol NO_3^-)	7 days	4–9 days	▶ No effect on exercise capacity (p=0.445) ▶ No effect on VO_2 (p=1.00) ▶ Significant higher in FeNO levels (p<0.001) ▶ Significant increase in plasma NO_3^- level (p<0.001) ▶ Significant increase in plasma NO_2^- level (p<0.001) ▶ No effect on SBP (p=0.482) ▶ No effect on DBP (p=1.000) ▶ No effect on HR p=0.191
Kerley *et al*, 2015/RXT[17]	N=11 GOLD stage 2–4 Age (y): 69±7 Sex (M/F): 5/6 FEV_1%=43.4	140 mL BRJ + 200 mL black currant cordial	140 mL water + 200 mL black currant cordial	Acute	7 days	▶ ISWT distance increased 25 m (p=0.005) ▶ Significant increase in plasma NO_3^- level (p=0.000005) ▶ Significant increase in plasma NO_2^- level (p<0.01) ▶ Significant change in SBP: 12 mm Hg (p=0.03) ▶ Significant change in DBP: 2 mm Hg (p=0.045) ▶ Significant change in MAP: 5 mm Hg (p=0.018) ▶ No effect on HR (p=0.19) ▶ No effect on O_2 saturation (p=0.71)
Kerley *et al*, 2019/RXT[18]	N=8 GOLD stage 1–3 Age (y): 63±7 Sex (M/F): 5/3 FEV_1%=55	140 mL BRJ (12.9 mmol)	140 mL ND-BRJ (0.5 mmol NO_3^-)	14 days	NA	▶ ISWT distance increased 56 m (p=0.0004) ▶ Significant increase in plasma NO_3^- level (p=0.015) ▶ Significant increase in plasma NO_2^- level (p=0.02) ▶ No effect on FeNO level (p=0.095) ▶ No effect on SBP (p=0.14) ▶ No effect on DBP (p=0.35)
Leong *et al*, 2015/RXT[31]	N=19 GOLD stage 2 Age (y): 67±8 Sex (M/F): 5/14 FEV_1%=62	140 mL BRJ (9.6 mmol)	140 mL ND-BRJ (0.0056–0.020 mmol NO_3^-)	3 days	4 days	▶ Endurance distance increased 79 m (p=0.494) ▶ Increase time to fatigue by 6% (p=0.693) ▶ Significant change SBP in safety phase: 10 mm Hg at 1-hour standing (p=0.001) and 7.5 mm Hg at 4-hour standing (p=0.029) ▶ No effect on DBP in safety phase: 0.1 mm Hg at 1-hour standing (p=0.966) and 2.7 mm Hg at 4-hour standing (p=0.352)

Continued

Table 1 Continued

Authors/design	Study sample	Nitrate (NO_3^-) dose	Placebo	Duration	Wash-out	Results (following NO_3^- vs placebo)
Pavitt *et al*, 2020/ RCT During PR[19]	N=122 GOLD stage 2–4 Age (y): 68±10 Sex (M/F): 69/53 FEV_1%=49	140 mL BRJ (12.9 mmol)	140 mL ND-BRJ	56 days	No	▶ Significant increase in ISWT distance by 60 m (p=0.027) ▶ Significant change in SBP: 5 mm Hg (p<0.0005) ▶ Significant change in DBP: 5 mm Hg (p<0.0005) ▶ Significant change in MAP: 5.0 mm Hg (p<0.0005) ▶ Significant change in the FMD in BRJ group +6.6% (p=0.046) ▶ Significant increase in physical activity by 348 step/day (p=0.02)
Shepherd *et al*, 2015/RXT[32]	N=13 GOLD stage 1–2 Age (y): 65±8 Sex (M/F): NR FEV_1%=57	140 mL BRJ (13.5 mmol)	140 mL ND-BRJ (0.004 mmol NO_3^-)	2.5 days	7 days	▶ No effect on 6MWD (p=0.17) ▶ No effect on baseline VO_2(p=0.56) and end exercise VO_2 (p=0.88) ▶ Significant increase in plasma NO_3^- level (p=0.002) ▶ No effect on SBP (p=0.91) ▶ No effect on DBP (p=0.25)

BRJ, beetroot juice; DBP, diastolic blood pressure; FeNO, fractional exhaled nitric oxide; FMD, flow-mediated dilatation; GOLD, Global Obstructive Lung Disease; ISWT, incremental shuttle walk test; MAP, mean arterial pressure; 6MWD, 6-minute walk distance; N, number of participants who completed the trial; NA, not available; NaCl, Sodium chloride; ND-BRJ, nitrate-depleted beetroot juice; NO_2^-, nitrite; NR, not reported; RCT, randomised controlled trial; RXT, randomised crossover trial; SBP, systolic blood pressure; VO_2, oxygen consumption.;

was reported in two studies; Curtis *et al* report a significant decrease in iso-time VO_2 after NO_3^- supplementation compared with a placebo (BRJ 16.6±6.0 mL/min/kg; placebo 17.2±6.0 mL/min/kg; p=0.043).[29] Differently, Berry *et al* failed to find lower iso-time oxygen uptake (median +IQR; BRJ 14.1+5.4; placebo 13.4+5.5; p=0.099).[16] We were unable to perform a meta-analysis of iso-time oxygen uptake due to incomplete data. Iso-time VO_2 and other cardiopulmonary exercise parameters are provided in (table 2).

Blood pressure

SBP and DBP were reported in all included studies, while MAP was reported in three studies.[17 19 29] Meta-analysis for systolic, diastolic and mean arterial blood pressure in people with COPD found significant reductions compared with placebo (figure 4) (MD (95% CI) was −3.39 mm Hg (−6.79 to 0.01), p=0.05, for SBP; −2.20 mm Hg (−4.36 to −0.03), p=0.05, for DBP; and −4.40 mm Hg (−7.49 to −1.30), p=0.005, for mean arterial blood pressure).[16–19 26 28–32] However, in one study in individuals with PHT, SBP and DBP did not significantly change following NO_3^- supplementation compared with placebo.[27]

Heart rate

The impact of NO_3^- supplementation on HR was reported in seven studies.[16 17 26–30] Pooled analysis from four trials of HR at rest and at peak of exercise in people with COPD is shown in figure 5. Following the meta-analysis, the MD (95% CI) was 0.23 (−3.58 to 4.03), p=0.91, for HR at rest and 0.22 (−5.80 to 6.24), p=0.94, for HR at peak of exercise, showing no change in HR following NO_3^- supplementation compared with placebo.[17 26 28 29]

Endothelial function

Pavitt *et al* assessed the impact of NO_3^- supplementation during PR on endothelial function using brachial artery flow-mediated dilatation,[19] finding an improvement

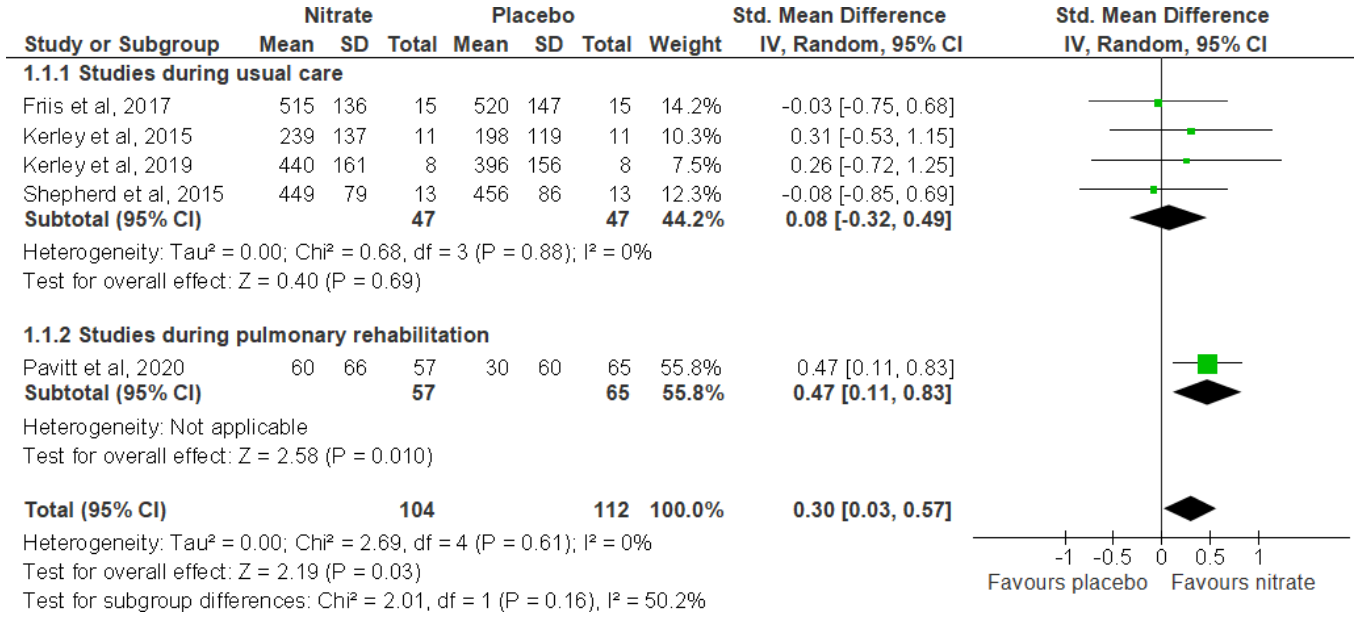

Figure 2 Forest plot for the effect of nitrate supplementation on peak exercise capacity measured with incremental shuttle walk test or 6-minute walk distance (in metres) in patients with chronic obstructive pulmonary disease.

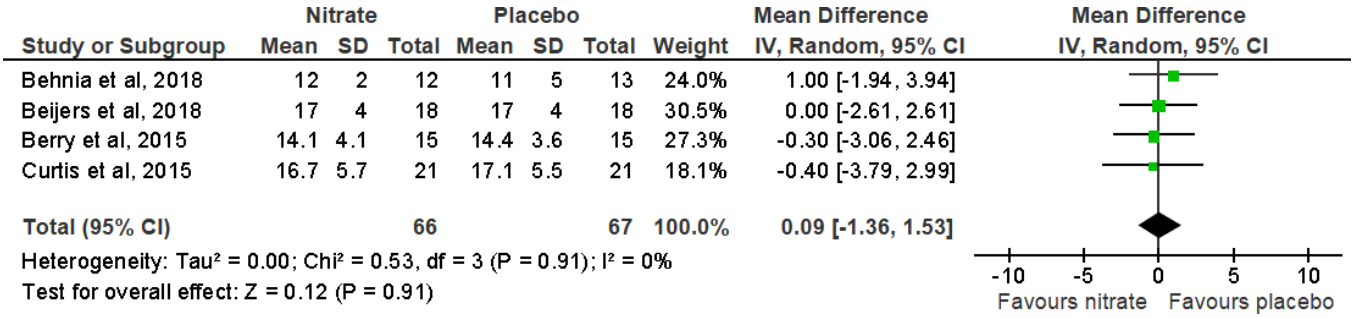

Figure 3 Forest plot for the effect of nitrate supplementation on peak oxygen consumption (mL/min/kg) in patients with chronic obstructive pulmonary disease.

(increase) in FMD in the treatment group (n=10) compared with placebo (n=10) (median (IQR) percent change: +6.6% (0.6 to 17.6), placebo: −4.7% (−21.5 to 11.8), and estimated treatment effect: −20.3% (95% CI −33.8 to 3.4); p=0.046).

O_2 saturation

The impact of dietary NO_3^- supplementation on O_2 sat was reported in three studies.[16 17 29] Pooled analysis from two trials for oxygen saturation at rest and at peak exercise in COPD is shown in figure 6.The MD (95% CI) was 0.20 (−1.72 to 2.12)%, p=0.84, for oxygen saturation at rest and −0.37 (−2.88 to 2.14)%, p=0.77, for oxygen saturation at peak of exercise, showing no effect on oxygen saturation following NO_3^- supplementation compared with placebo.[17 29] Curtis *et al* did demonstrate a reduction in area under the curve for oxygen saturation during exercise with NO_3^- supplementation compared with placebo.[29] Of note, this study excluded individuals with resting hypoxia.

Plasma NO_3^- and NO_2^- levels

Plasma NO_3^- and NO_2^- levels were measured in seven studies.[16–18 26 27 29 32] Pooled analysis from six trials for plasma NO_3^- and NO_2^- levels in COPD individuals is shown in figure 7. Following the meta-analysis, the MD (95% CI) was 445.61 (254.69 to 636.53), p<0.00001, for plasma NO_3^- level and 367.07 (232.87 to 501.27), p<0.00001, for plasma NO_2^- level, showing that levels of plasma NO_3^- and NO_2^- significantly increased following NO_3^- supplementation compared with placebo.[16–18 26 29 32]

Fractional exhaled NO

The impact of NO_3^- supplementation on FeNO was measured in three trials two conducted in individuals with COPD[18 28] and one with PHT.[27] Pooled analysis from two trials for FeNO in COPD individuals is shown in figure 8. Following the meta-analysis, the MD (95% CI) was 17.23 (−3.35 to 37.80) ppb, p=0.10, for FeNO, showing no consistent effect on FeNO following NO_3^- supplementation compared with placebo.[18 28] However, Henrohn *et al* (2018) found that the level of FeNO in individuals with PHT increased at all flow rates (50–300 mL/s) following NO_3^- supplementation compared with placebo, at a flow rate of 50 mL/s (median of differences 18, 95% CI 11 to 26, p<0.0010).[27]

Risk of bias and evidence quality assessment

Using the Cochrane risk-of-bias assessment tool,[33] the included studies showed considerable variation in the risk of bias, but most were limited by a lack of allocation concealment, blinding and incomplete reporting of data (figure 9).

The Grading of Recommendations, Assessment, Development and Evaluations (GRADE)[34] criteria were used

Table 2 Iso-time oxygen saturation and other cardiopulmonary exercise parameters

Study	Parameter	Placebo	BRJ	P value
Berry *et al*, 2015[16]	HR	112 (99, 124)	110 (97, 123)	0.300
	SBP	167.1 (151.7, 182.4)	160.1 (147.8, 172.5)	0.137
	DBP	86.3 (79.6, 92.9)	79.9 (72.8, 86.9)	0.001
	VO_2*	13.4+5.5	14.1+5.4	0.099
	O_2 saturation	95.1 (94.0, 96.2)	95.1 (93.9, 96.2)	0.895
Curtis *et al*, 2015[29]	HR	122 (17)	121 (20)	0.30
	VO_2	17.2 (6.0)	16.6 (6.0)	0.043
	O_2 saturation	92 (4)	93 (4)	0.15

Berry *et al*, 2015; Curtis *et al,* 2015: Data are presented as mean (SD).
*Non-normally distributed variables are presented as medians and IQRs. Normally distributed values are presented as means and 95% CIs.
BRJ, beetroot juice; DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure; VO_2, oxygen comsumption.

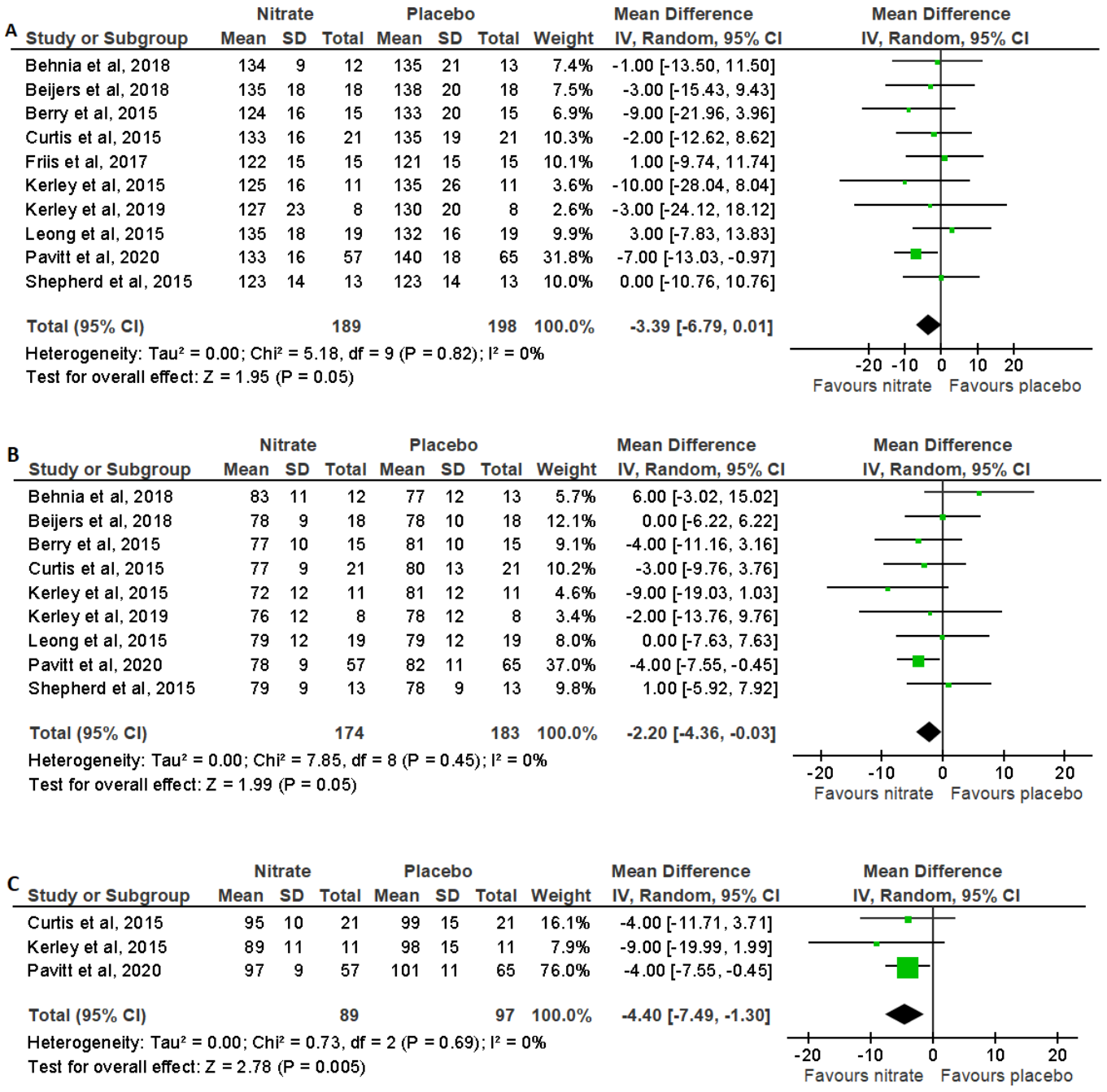

Figure 4 Forest plot for the effect of nitrate supplementation on (A) systolic blood pressure (mm Hg), (B) diastolic blood pressure (mm Hg) and (C) mean arterial blood pressure (mm Hg) in patients with chronic obstructive pulmonary disease.

to assess the overall evidence around specific outcomes (eg, exercise capacity and blood pressure endpoints). For exercise capacity, the majority of studies were small and short term, which limit the precision of estimates. Studies did not focus clearly on disease severity, limiting the directness of the evidence to COPD phenotypes, particularly hypoxic patients. Further, studies used a variety of interventions (eg, doses, duration and delivery method) and outcome measures contributing to heterogeneity or inconsistency. Therefore, the quality of evidence by GRADE to support an effect of NO_3^- supplementation on exercise capacity in people with lung disease is low. In the specific context of PR, only a single high-quality RCT, the largest and the longest of the included studies, was identified. Therefore, the total evidence by GRADE to support the impact of NO_3^- supplementation on exercise capacity in the context of PR is moderate. Regarding blood pressure endpoints, studies were consistent, although the longest duration study is only 8 weeks, so taken together, the evidence to support an impact of NO_3^- supplementation on blood pressure is moderate.

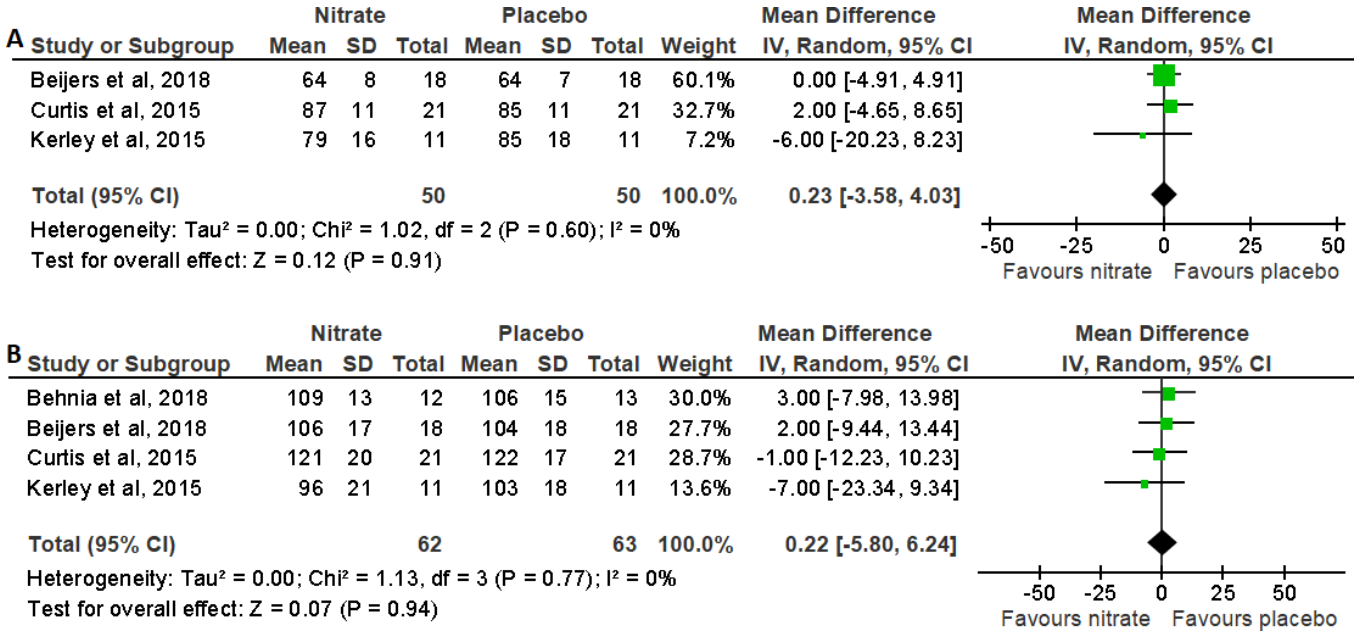

Figure 5 Forest plot for the effect of nitrate supplementation on (A) heart rate at rest (bpm) and (B) heart rate at peak (bpm) in patients with chronic obstructive pulmonary disease.

DISCUSSION

The main findings of this review into the effects of dietary NO_3^- supplementation in people with CRD are that, although it can augment the effects of PR on exercise performance, a consistent short-term effect on exercise capacity in the absence of exercise training has not so far been demonstrated. However, studies to date do suggest that dietary NO_3^- supplementation can lower blood pressure, perhaps by improving endothelial function, which is potentially important given the high prevalence of cardiovascular disease in people with COPD, especially if a single intervention could address both issues.

Significance of findings

NO is a vital physiological mediator in the body. It is produced in two different ways: by an endogenous pathway (oxygen-dependent) via the L-arginine NO synthase system and by an exogenous pathway (oxygen-independent) via the reduction of dietary NO_3^- via NO_2^- to NO.[35 36] In the human diet, the main source of NO_3^- is green leafy vegetables, which have high concentrations of NO_3^-. NO_3^- reduction to NO is favoured by conditions found in exercising muscle, in particular hypoxia, acidosis and the presence of deoxyhaemoglobin and myoglobin. Effects on exercise in people with respiratory

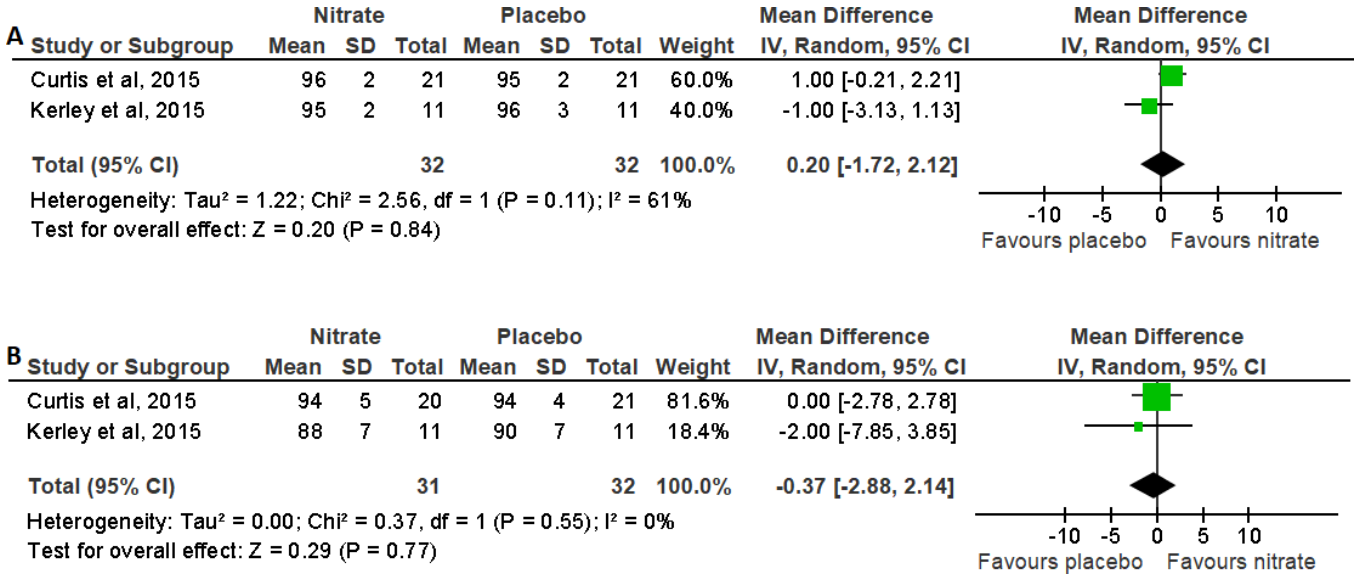

Figure 6 Forest plot for the effect of nitrate supplementation on (A) oxygen saturation at rest (%) and (B) oxygen saturation at peak (%) in patients with chronic obstructive pulmonary disease.

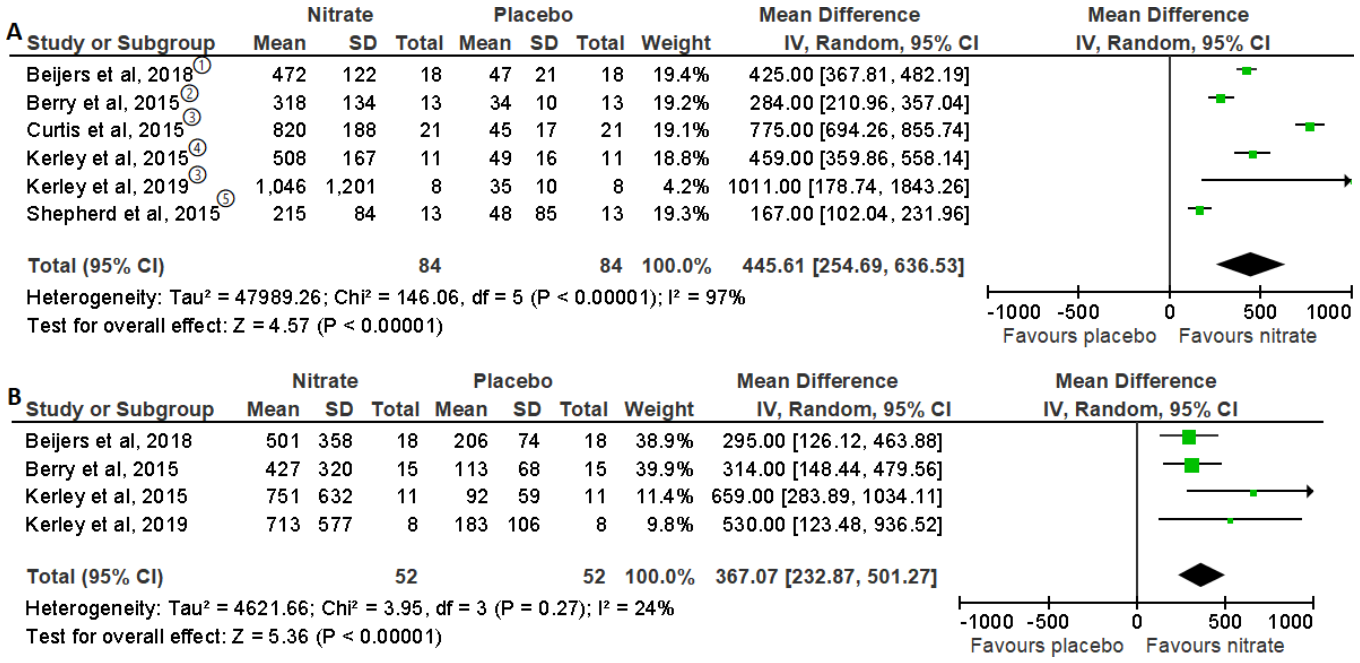

Figure 7 Forest plot for the effect of nitrate supplementation on (A) plasma nitrate (μM) and (B) plasma nitrite (nM) levels in patients with chronic obstructive pulmonary disease. (1) sodium nitrate (8 mmol); (2) beetroot juice (BRJ) (7.58 mmol); (3) BRJ (12.9 mmol); (4) 140 mL of BRJ + 200 mL black currant cordial; (5) BRJ (6.75 mmol).

disease could be mediated through improved muscle mitochondrial efficiency or through effects on vascular endothelium in either the systemic or pulmonary circulation. Endothelial effects of NO are also likely to underpin the effects on blood pressure that were observed.

Regarding the impact of dietary NO_3^- supplementation on exercise capacity, the current meta-analysis includes studies in COPD, which found an improvement in exercise capacity,[16–19] and others that did not.[26 28–32] Studies included heterogeneous COPD populations (eg, COPD severity and age) and used different exercise protocols (eg, ISWT, 6MWD and endurance time during cycle ergometry). Furthermore, hypoxic patients who required oxygen supplementation, a patient phenotype that might be expected to benefit most given that NO_2^- to NO conversion is enhanced in hypoxic conditions, were typically excluded. The duration of treatment and doses of NO_3^- used in trials also differed. The results from trials indicate that longer-term studies in specific patient phenotypes are needed to see if NO_3^- supplementation can improve exercise capacity in the absence of a training stimulus. Likewise, although dietary NO_3^- supplementation was associated with a greater increase in walk distance during PR, it is not clear how long this benefit might be sustained for—the 8-week ON-EPIC trial[19] is the longest study to date of this intervention in people with CRD.

Physiological parameters at peak exercise including VO_2 did not change significantly with NO_3^- supplementation compared with placebo,[16 26–30 32] although one study found a significant reduction in VO_2 at iso-time during cycle exercise in patients with COPD.[29] Again, these negative results could be due to an absence of effect or a result of using insufficient dose or duration of supplementation. A dose–response effect for reduction in VO_2 during exercise has previously been described in healthy individuals.[37]

Dietary NO_3^- supplementation has been shown to reduce blood pressure in individuals who are either normotensive[38] or hypertensive.[39] We found an overall effect to lower systolic, diastolic and mean arterial blood pressure in the studies reviewed here. People with lung disease are at high risk of cardiovascular disease, and this

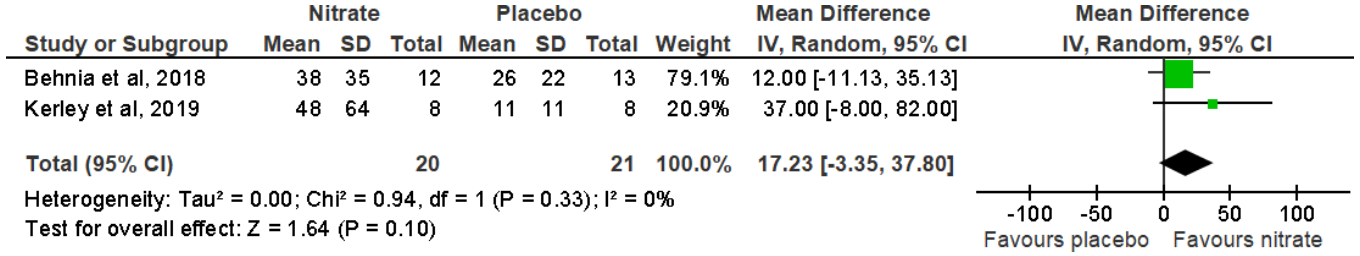

Figure 8 Forest plot for the effect of nitrate supplementation on fractional exhaled nitric oxide (ppb) in patients with chronic obstructive pulmonary disease.

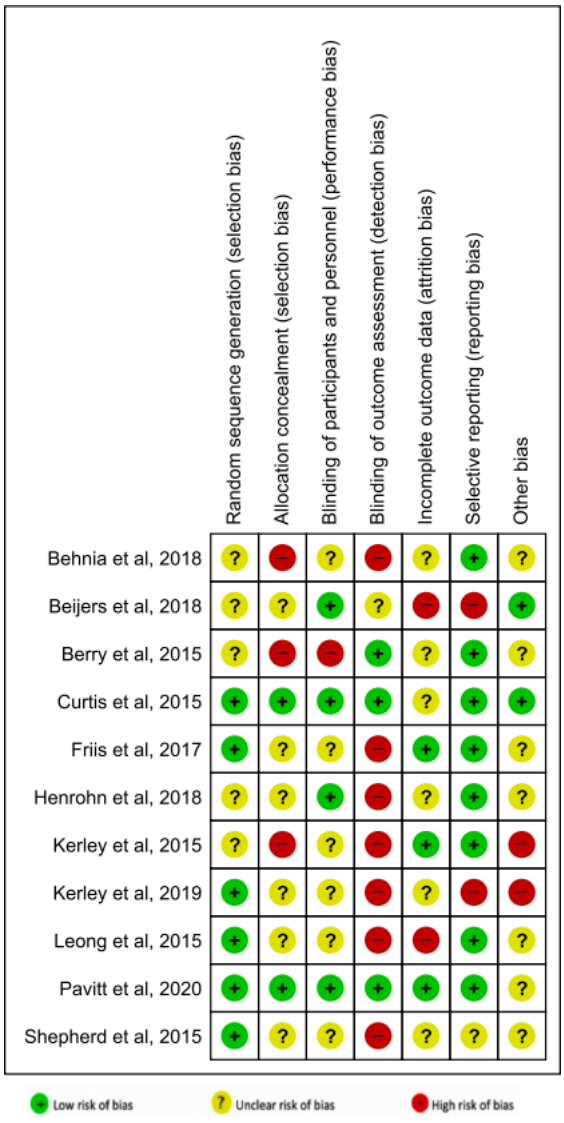

Figure 9 Risk of bias summary: review authors' judgements about each risk-of-bias item for each included study.

includes damage to the pulmonary vascular bed, which can lead to PHT.[40] There is also interesting data in individuals with idiopathic PHT, which demonstrate that a low level of plasma NO_3^- is associated with increased mortality risk making it a potential prognostic indicator for PHT.[41] Although one study with PHT was identified by our search strategy to be small, we advise against over-interpreting it, and further studies are needed.

Plasma NO_3^- and NO_2^- levels have potential to be used as a biomarker for NO availability.[42] As expected, the available evidence showed that plasma NO_3^- and NO_2^- levels increased following dietary nitrate supplementation. The FeNO has been used as a diagnostic test for asthma.[43 44] However, studies describing the FeNO level in people with COPD are inconclusive. In this review, two studies showed that FeNO level increased following NO_3^- supplementation,[27 28] while another study had a negative result,[18] so further work is needed to clarify this. Further work is needed to establish if FeNO can be used as a biomarker to monitor compliance in therapeutic trials of NO_3^- supplementation or even to adjust dose in individuals.

Strengths and limitations

A variety of lessons can be learnt from this review. First, most of the trials covering the effect of dietary NO_3^- supplementation on exercise capacity in people with respiratory disease have focused on COPD, with only one on PHT. Most trials were short term. Second, trials involved a variety of study designs, outcome measures, clinical phenotypes (severity of the disease and of hypoxia in particular), exercise protocols and dose and duration of NO_3^- supplementation. Third, it will be important to define whether there are different COPD phenotypes or subpopulations that can be categorised as NO_3^- responders or non-responders. Fourth, in most studies, BRJ was used as the source of NO_3^-. It is possible that other bioactive compounds in the juice that have antioxidant and anti-inflammatory properties including vitamin C, carotenoids, phenolics and betalains could contribute to beneficial effects. Many but not all studies have used NO_3^--depleted BRJ as a control, which is ideal for identifying effects of NO_3^- itself but runs the risk of underestimating the effect of BRJ itself if these other components have a role. Finally, none of the trials we identified for this review evaluate the effect of dietary NO_3^- supplementation on exercise capacity or cardiovascular parameters in people with rarer lung diseases such as ILD and cystic fibrosis.

CONCLUSION

Dietary NO_3^- supplementation has potential to reduce cardiovascular risk by lowering blood pressure in people with COPD as well as improving exercise capacity, though evidence for the latter is largely in the context of PR. Importantly, further work is needed to understand whether it is the rehab setting that is giving the benefit (ie, combining supplementation with exercise) or whether it is purely due to the fact that participants were followed for a longer duration than in other non-PR studies. To date, no data exist that might support dietary NO_3^- supplementation for lung diseases other than COPD. The data support trials of dietary NO_3^- supplementation in patients with COPD to address vascular endpoints, and we would suggest that exercise capacity also be measured in such trials to see whether a 'dual benefit' can be elicited. Outside the context of PR, further trials are indicated to evaluate the value of dietary NO_3^- supplementation on exercise capacity in COPD and specifically to identify the phenotypes most likely to profit from this intervention.

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